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(19) (CA) **CANADIAN PATENT** (12)

(54) Apparatus for the Parallel Performance of a Plurality of Chemical Reaction Sequences

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This invention relates to apparatus for the parallel performance of a plurality of chemical reaction sequences. Apparatus of this kind is suitable particularly for the synthesis of polymer molecules, such as nucleotides, proteins, etc. on a carrier material such as, for example, glass, silica gel or some other suitable material.

The chemical synthesis of such polymer molecules is usually carried out in reaction chambers (e.g. frits, columns, etc.) in which the first building block of the molecule for synthesis is present in a form in which it is fixed to the corresponding polymer material and the reagents required for synthesis are added either manually or automatically.

It is in the nature of the reaction sequences that the synthesis of a relatively long-chain molecule takes a relatively long time. If a plurality of different polymer molecules synthesized from the same building blocks are required, then the time taken until

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all the required synthesis products are available is often unacceptably long. There is therefore a need for a possibility of simultaneously synthesizing a plurality of different polymer molecules from the same reagents. In this sense the object of the invention is to provide apparatus with which a plurality of reaction sequences can be performed simultaneously.

SUMMARY OF THE INVENTION

Thus the present invention provides an apparatus for performing chemical reaction sequences, characterised by: a first number n of reaction discs which are superposed in the form of a stack and are individually displaceable relatively to one another, means for defining the displacement in a second number m of identical steps, m continuous bores disposed in the discs at the step distances from one another, one of the bores being widened out per disc to form a reaction chamber, means for retaining a support material in the reaction chamber on the flow of a reagent therethrough; and concentric grooves in the undersides of the reaction discs around the bores, the grooves containing O-rings.

The apparatus is intended more particularly for the simultaneous synthesis of a plurality of DNA and RNA segments of different chain lengths and sequence on a polymer support material. In these conditions the DN segments are synthesized in known manner from mononucleotides, it being possible to use any desired suitable support material, preferably glass particles, and various synthesis strategies, preferably the tri-ester or the phosphite tri-ester process.

One exemplified embodiment of the invention will be described hereinafter with reference to the accompanying drawings wherein:

Fig. 1 is a partial section of an apparatus with ten reaction discs, shown in perspective.

Fig. 2 is a top plan view of a single reaction disc.

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Fig. 1 illustrates an apparatus for the simultaneous synthesis of ten different DNA segments, the support used being a polymer granulate. The first building block of the chains to be synthesized is already disposed on the support before the synthesis starts.

The apparatus consists of a stack of concentrically superposed round discs, which are formed with a plurality of duct systems. The reaction discs 1 - 10 are associated with the ten oligonucleotides for synthesis, i.e. a specific oligonucleotide is synthesized in each reaction disc. To this end, for example, the first reaction disc 1 has a reaction chamber 11 in the form of a bore disposed approximately midway between the axis and the edge of the disc and extending from the top surface to about three-quarters of the disc thickness. The chamber is intended to receive the support granulate. At the top edge the chamber has a peripheral widening to receive a frit 12, which closes the reaction chamber 11 at the top. Another frit 13 is provided in a concentri recess in the bottom of the chamber and forms the bottom closure for the chamber 11 with respect to a continuous bore of very small diameter, e.g. 1 mm, leading from the bottom of the chamber to the underside of the disc.

In addition to the reaction chamber 11, the reaction disc 1 has four continuous bores 14 of the same small

diameter, e.g. 1 mm. These bores are offset by 72°, 144°, 216° and 288° respectively from the reaction chamber.

The other reaction discs are constructed in the same way. They are individually rotatable relatively to one another about a central connecting element.

The immediately adjacent reaction disc 2 is shown turned through 144° in the drawing so that its reaction chamber 15 is situated coaxially of the bore 14 of the first disc 1.

The next reaction disc 3, which is not shown in section in the drawing, is again in the position in which its reaction chamber - which will be seen from the frit 16 - is in alignment with the reaction chamber 11 of the disc 1. A bore 17 offset by 72° can be seen at disc 3.

The stack of reaction discs 1 - 10 is bounded by a top connecting disc 18 for connecting the reagent feed hoses and a bottom connecting disc 21 for connecting the discharge hoses. The top connecting disc 18 has screwthreaded bores 19 in line with the ducts and reaction chambers in the reaction discs to receive fittings for the feed hoses. The bores 19 which extend approximately to three-quarters of the disc thickness continue, as in the case of the reaction chambers, in the form

of continuous narrow bores as far as the underside of the plate. The bottom connecting disc 21 may be either identical to the top disc or else have a single collecting duct (not shown) if the spent reagents do not have to be discharged separately.

On their undersides, the reaction discs 1 - 10 and the connecting disc 18 have grooves which enclose the narrow bores in the form of a ring to receive O-rings by means of which the ducts are sealed at the transitions between the discs. These sealing systems have been omitted from the drawing in order not to overload it.

A top biasing disc 20 is disposed above the top connecting disc 18 and similarly a bottom biasing disc 22 is provided beneath the bottom connecting disc 21. These discs 20, 22 transmit to the stack the force produced by a bolt 23 acting as the central connecting element and extending through an axial bore passing through the entire stack, said bolt having a screw-threaded connection (nut 24). This biasing force so presses the discs upon one another as to guarantee absolute sealing-tightness of the ducts and eliminate any possible dead space inside the sealing rings.

Appropriate alignment of the reaction discs 1 - 10 causes their reaction chambers and bores to be

so aligned that four ducts are formed in the stack, each of these now being fed with one of the mononucleotides A, T, C or G (denoted by arrows in the drawing) so that the growing DNA fragments are simultaneously lengthened by the corresponding nucleotide.

To this end, the reaction chamber 11 of disc 1 is brought for the appropriate time into the duct whose nucleotide is being added (in the drawing it is in the duct in which T is being introduced, while the chamber of disc 2 is in the C-duct in which, therefore, C is added on in the same time as T in the case of disc 1. All the reactions and washing processes required for the addition of the mononucleotides (buffer group separation, capping, and possibly oxidation) also take place simultaneously (continuous flow process). On completion of an addition cycle, the individual discs are so rotated that their chambers are in the duct of the next nucleotide to be added. In order to turn the discs, nut 24 is released and then re-tightened. Once the synthesis of a DNA fragment is completed in one of the discs, its chamber is set to the empty position, the remaining four narrow bores of the disc maintaining the four connecting ducts for the extension of the DNA segments in the other reaction discs.

On conclusion of the synthesis of all the DNA segment

in the individual reaction discs, the apparatus is dismantled and the frit 12 removed whereafter the support material with the synthesized nucleotide sequence adhering is removed. After the buffer groups have been separated and released from the support material the required unbuffered DNA segments are obtained in pure form from the crude mixtures by a suitable purification process.

In order that the individual rotation of the individual reaction discs may be confined to the appropriate angular steps of 72°, at least visible markings must be provided. It is, however, preferable to provide a click-stop mechanism for the correct angular settings.

The apparatus may be operated manually or mechanically with suitable drive means. The latter operation can also be combined with a program control.

The apparatus described as an example above consists of reaction discs of a diameter of about 60 mm and a thickness of about 10 mm. As already stated, the bores 14 have a diameter of about 1 mm. The reaction chambers have a diameter of about 6 mm. These dimensions are, however, relevant only in connection with a specific synthesis program. Apparatus having the same synthesis and functional principle may be of dimensions which can be selected within wide limits and, in particular,

be much larger than the example described. The number of bores per disc can be larger than the above example. This is important, for example, in the case of peptide synthesis.

The shape of the discs and, in particular, the reaction chambers is, of course, in no way limited to the exemplified embodiment. It would be possible, for example, to replace the circular arrangement of bores on circular discs by a linear arrangement of bores and accordingly provide a linear displacement of the discs instead of rotation.

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS

1. Apparatus for performing chemical reaction sequences, characterised by:

a first number n of reaction discs which are superposed in the form of a stack and are individually displaceable relatively to one another,

means for defining the displacement in a second number m of identical steps,

m continuous bores disposed in the discs at the step distances from one another, one of the bores being widened out per disc to form a reaction chamber,

means for retaining a support material in the reaction chamber on the flow of a reagent therethrough;

and concentric grooves in the undersides of the reaction discs around the bores, the grooves containing O-rings.

2. Apparatus according to claim 1, characterised in that the reaction discs are circular and rotatable relatively to one another and the bores are also disposed on them in the form of a circle.

3. Apparatus according to claim 2, characterised in that the step distances are identical angle steps.

4. Apparatus according to claim 1, characterised in that the means for retaining the support material in the reaction chamber are frits disposed in appropriate recesses in the reaction chamber.

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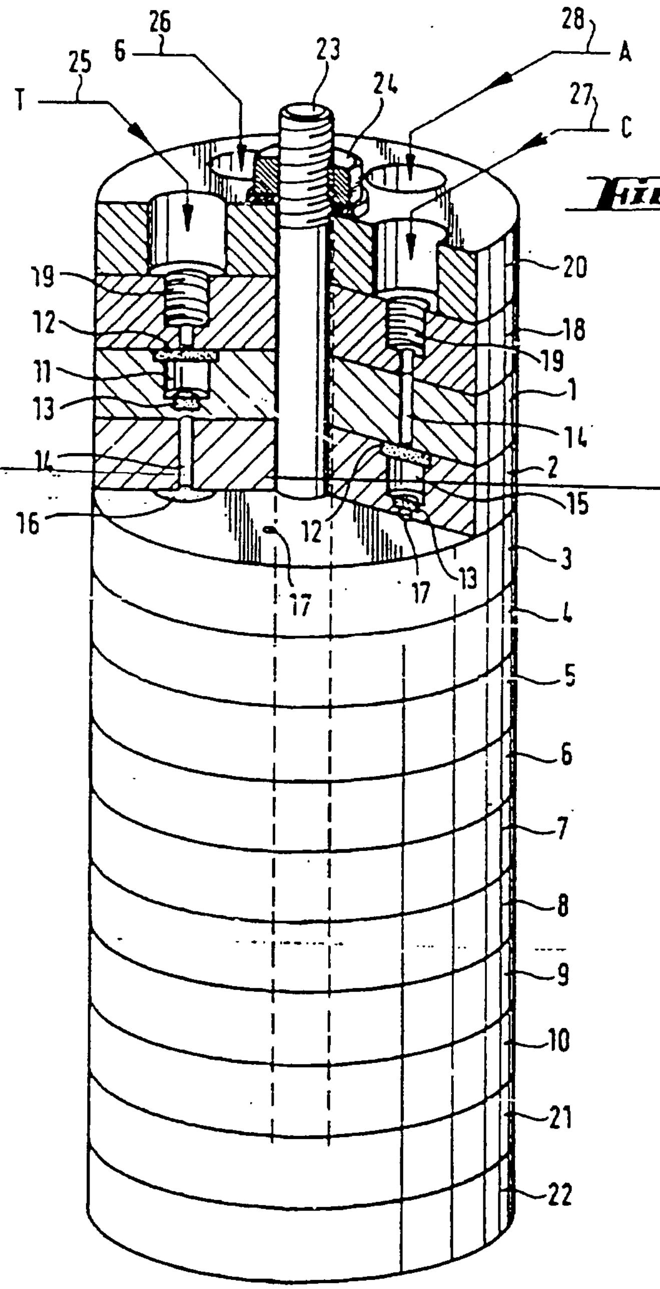

Fig. 1

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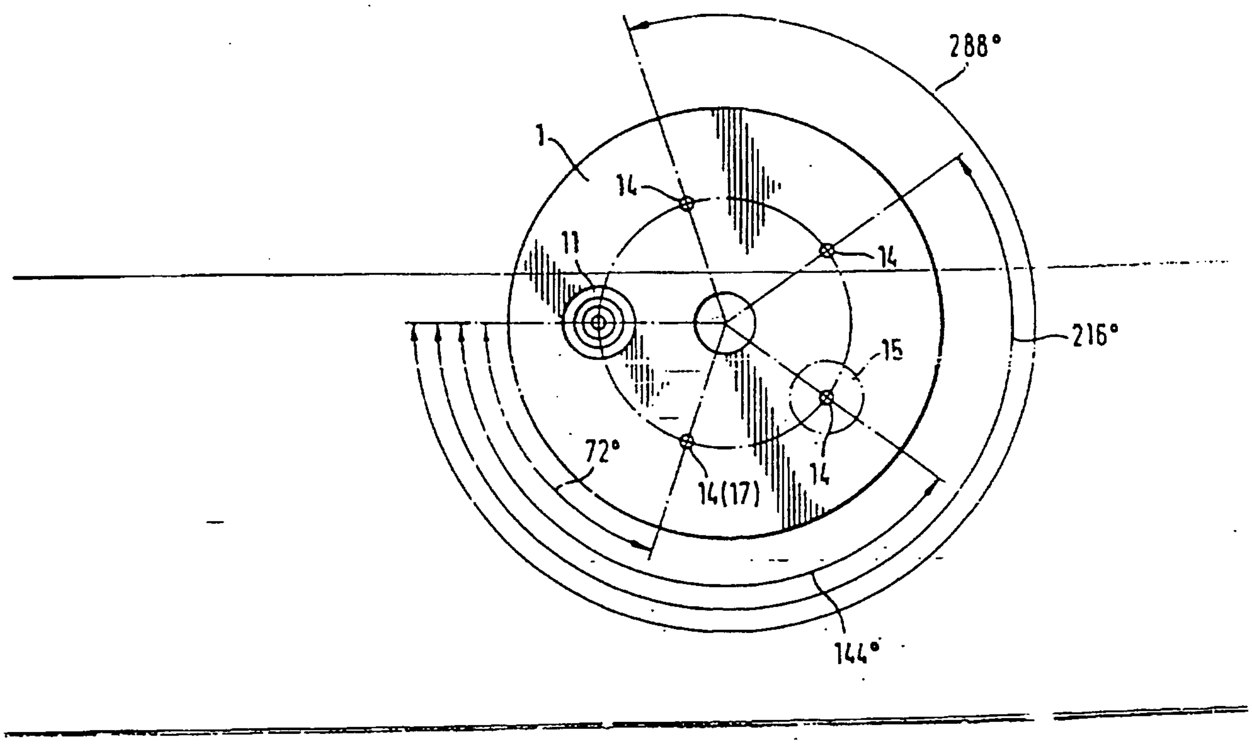

Fig. 2

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